

## Mortality and Repellency Effect of Some Plant Oils against *Tribolium castaneum* (Coleoptera: Tenebrionidae)

Comment [K1]: Essential Oils

### ABSTRACT

The red flour beetle (*Tribolium castaneum*) is a major pest of stored grains, causing significant economic losses. In recent years, essential oils have gained attention as eco-friendly alternatives to synthetic pesticides. This study evaluated the insecticidal and repellent properties of three essential oils—lavender (*Lavandula spica*), mogra (*Jasminum sambac*), and rose (*Rosa damascena*)—against *T. castaneum*. Toxicity was assessed using direct contact and fumigation methods, targeting adult *T. castaneum*, respectively. Repellency was tested on larvae using filter paper assays. Essential oils were procured from India, and bioassays were conducted at varying doses and exposure durations. Mortality data were corrected using Abbott's formula and analyzed using probit analysis, while repellency effects were evaluated using ANOVA. In direct contact assays, adult mortality increased with higher doses and longer exposure times. Lavender oil showed the highest toxicity ( $LD_{50} = 0.366\text{--}0.143 \mu\text{l}/\text{cm}^2$ ), followed by Mogra oil ( $LD_{50} = 1.037\text{--}0.147 \mu\text{l}/\text{cm}^2$  at 3–48 h) and rose oil ( $LD_{50} = 0.651\text{--}0.188 \mu\text{l}/\text{cm}^2$ ). In fumigation assays, rose oil was the most effective against the target insect ( $LD_{50} = 4.031\text{--}2.252 \mu\text{l}/\text{cm}^2$  at 24–48 h), followed by lavender oil ( $LD_{50} = 4.980\text{--}3.180 \mu\text{l}/\text{cm}^2$ ) and mogra oil ( $LD_{50} = 7.339\text{--}5.463 \mu\text{l}/\text{cm}^2$ ). Overall, the direct contact method was more effective than fumigation. Repellency assays showed mogra oil exhibiting significant effects ( $F = 25.25$ ,  $P < 0.001$ ) and achieving 100% repellency at  $0.0785 \mu\text{l}/\text{cm}^2$  within 3–4 hours, whereas lavender and rose oils showed lower and statistically insignificant effects. This study highlights the potential of essential oils as biopesticides for managing *T. castaneum*. Mogra oil demonstrated superior efficacy in direct contact assays, while rose oil excelled in fumigation tests. These findings support the use of essential oils as eco-friendly alternatives for pest management in stored grains.

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**Keywords:** Lavender, Mogra, Rose, essential oils, toxicity, Repellent activity, Fumigation, *Tribolium castaneum*,  $LD_{50}$  values.

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## INTRODUCTION

The exponential growth of the global human population, projected to exceed 10 billion by 2050 (FAO, 2017), has intensified the need for food security worldwide. This challenge is particularly acute in developing and underdeveloped regions, where post-harvest losses due to insect infestations significantly reduce the availability of stored grains and other staples. Among stored-product pests, the red flour beetle (*Tribolium castaneum*), a cosmopolitan species, is particularly destructive, causing extensive damage to grains, flours, and other stored products (Pimentel et al., 2007). Infestations lead to both qualitative and quantitative losses, with significant economic implications.

Chemical control, predominantly through synthetic pesticides and fumigants, has been the mainstay for managing *T. castaneum* and other storage pests. However, these methods pose significant challenges, including environmental pollution, pesticide resistance, human toxicity, and residue accumulation in food products (Isman, 2006; Watts and Williamson, 2015). The need for sustainable, eco-friendly alternatives to synthetic pesticides has never been more urgent.

Plant-derived essential oils (EOs) are emerging as promising biopesticides due to their multifaceted mode of action, including toxicity, repellency, and disruption of insect development and reproduction (Rajendran and Sriranjini, 2008; Campolo et al., 2018). Essential oils are volatile, aromatic compounds produced through secondary metabolism in plants, with known insecticidal and repellent properties (Kim et al., 2012). Studies have demonstrated their efficacy against a variety of stored-product pests, such as *Callosobruchus chinensis*, *Rhyzoperthadominica*, and *Sitophilus zeamais* (Ukeh et al., 2009; Sharaby et al., 2009).

Essential oils from the families Lamiaceae and Apiaceae, such as lavender (*Lavandula spica*), rose (*Rosa damascena*), and jasmine (*Jasminum sambac*), have shown promising insecticidal and repellent activity in previous research (Wang et al., 2019; Sharaby et al., 2009). The use of such natural products aligns with the global push toward sustainable agricultural practices, reducing reliance on synthetic chemicals while maintaining efficacy against storage pests.

This study aims to evaluate the toxicant, fumigant, and repellent properties of three essential oils—mogra (*J. sambac*), lavender (*L. spica*), and rose (*R. damascena*)—against adult and larval stages of *T. castaneum*. By analyzing the response of this key storage pest to varying

concentrations of these oils, the research seeks to contribute to the development of safer, plant-based alternatives to conventional insecticides. A better understanding of the behavioral and toxicological effects of these essential oils on storage pests will help pave the way for integrated pest management strategies that minimize environmental and health risks while addressing food security concerns.

## MATERIALS AND METHODS

### Insect (Rearing and Maintenance)

The test insect, *Tribolium castaneum* (red flour beetle), was obtained from stock cultures maintained at the Entomology Laboratory, University of Rajshahi, Bangladesh. Adults were reared in glass beakers containing sterile feeding media consisting of whole-wheat flour and dried yeast powder in a 19:1 ratio, following the method described by Zyromska-Rudzka (1966). The wheat flour was stored in a deep freezer for 48 hours before use to eliminate any pre-existing pests. Subcultures of adult beetles were maintained at ambient laboratory conditions and sieved periodically to collect individuals for the experiments. Adults were manually transferred from the flour medium using a brush and spoon, then placed in Petri dishes for subsequent assays.

Comment [K6]: The red flour beetle

### Essential Oils

Pure essential oils of lavender (*Lavandula spica*), mogra (*Jasminum sambac*), and rose (*Rosa damascena*) were purchased from markets. These oils were used without further purification.

### Contact Toxicity Bioassay

A contact toxicity bioassay was performed using newly emerged *T. castaneum* adults (4–7 days old). Doses of each essential oil (0.331, 0.214, 0.136 and 0.097  $\mu\text{l}/\text{cm}^2$ ) were determined through pilot studies to ensure mortality rates between 10% and 90%. Oils were diluted in acetone, and 1 ml of the prepared solution was applied to a 7 cm Petri dish using a residual film method (Busvine, 1960). The solution was evenly spread across the surface, and the dishes were allowed to air dry. Control dishes received only acetone.

For each replication, 10 adults were introduced into the treated dishes and covered with lids to create a captive environment. Mortality was recorded at 3, 6, 24, and 48 hours post-treatment. Three replicates were conducted for each dose.

### Fumigation Toxicity Bioassay

The fumigant toxicity assay was conducted using glass vials (5 cm length  $\times$  2.5 cm diameter) capped with polypropylene stoppers. Groups of 10 adult beetles were placed into each vial, which was sealed with muslin cloth secured by adhesive tape. Doses of essential oils (4.074, 2.037, 1.018 and 0.509  $\mu\text{l}/\text{cm}^2$ ) were applied separately to identical vials, and these were

inverted and sealed with the insect-containing vials to allow saturation of the airspace with oil vapors. Controls were prepared using the same setup without essential oils. Mortality was assessed at 24 and 48 hours after treatment.

### **Repellency Bioassay**

Repellency was tested in 9 cm Petri dishes divided into treated and untreated halves using filter paper. Essential oils were applied to the treated half at concentrations of 0.0049, 0.0098, 0.0196, 0.0392, and 0.0785  $\mu\text{l}/\text{cm}^2$ , while the untreated half received acetone. A thin stick secured with adhesive tape demarcated the two sections. Ten fourth-instar larval beetles were introduced at the center of each dish, and their distribution was recorded hourly for 5 hours. Each concentration was tested in triplicate.

### **Statistical Analysis:**

#### **Dose Mortality Analysis**

Mortality data were corrected using Abbott's (1925) formula to account for natural mortality in the control:

$$P_t = \frac{P_o - P_c}{100 - P_c} \times 100$$

Where  $P_t$  = Corrected mortality (%),  $P_o$  = Observed mortality (%),  $P_c$  = Control mortality (%). Then mortality data were subjected to probit analysis (Busvine, 1971) to determine dose-mortality relationships.

#### **Repellency Analysis**

Percentage repellency (PR) was calculated using the formula:

$$PR = (NC - NT) / (NC + NT) \times 100$$

Here, PR= Percentage Repellency, NC= Number of insect in the non-treated (control) area after the exposure interval, NT= Number of insect in the treated area after the exposure interval (Nerio et al., 2009; Obeng-Ofori, 1995). Positive PR values indicate repellency, while negative values indicate attraction.

A two-factor without replication ANOVA was performed in Microsoft Excel to evaluate the effects of dose and time on repellency. The F-values were compared with critical values from the F-distribution table to determine statistical significance.

## RESULTS

### Contact Toxicity:

The doses of essential oil (Lavender, Mogra, Rose) used in contact treatment were 0.331, 0.214, 0.136 and 0.097  $\mu\text{l}/\text{cm}^2$ . The results on the mortality of *T. castaneum* adults after 3, 6, 24 and 48 hour of exposure to Lavender, Mogra and Rose essential oils are presented in Table 1.

LD<sub>50</sub> value of the lavender oil after 3 hour was 0.366  $\mu\text{l}/\text{cm}^2$  and after 48 hour of exposure period it became 0.143  $\mu\text{l}/\text{cm}^2$ . Though the LD<sub>50</sub> value were decreases by the time period so it's toxicity level was increasing. The lavender becoming more toxic at 48 hour of treatment for the test insect against *T. castaneum*. LD<sub>50</sub> value of the Mogra oil after 3, 6, 24 and 48 hour of exposure period were 1.037, 0.295, 0.185 and 0.147  $\mu\text{l}/\text{cm}^2$ . By the time period LD<sub>50</sub> value were decreasing and becoming toxic against *T. castaneum*. LD<sub>50</sub> value of the Rose oil after 3, 6, 24 and 48 hour of exposure period were 0.651, 0.503, 0.339 and 0.188  $\mu\text{l}/\text{cm}^2$ . So after 48 hour of exposure time period the LD<sub>50</sub> value becoming low and confirm their increasing toxicity level. From above discussion it is confirmed that among the three essential oils viz Lavender, Mogra and Rose oil ; the lavender oil was relatively more toxic than other two oils compare to their LD<sub>50</sub> value. Among the three oils lavender oil shows highest mortality rate at the highest doses. After 48 hour of exposure time the LD<sub>50</sub> value of three oils were 0.143, 0.147 and 0.188  $\mu\text{l}/\text{cm}^2$ , so at this point the highest toxicity rate compare to LD<sub>50</sub> value were Lavender oil > Mogra oil > Rose oil.

**Table 1:** LD<sub>50</sub>, 95% confidence limits and regression equation of Lavender, Mogra and Rose essential oil against adult *T. castaneum* after 3, 6, 24 and 48 hours of contact treatment.

Time of exposure	Oils	LD <sub>50</sub>	95% confidence limits		Regression equation	$\chi^2$ value (at 2df)
			Lower	Upper		
3h	Lavender	<b>0.366</b>	0.228	0.588	$Y = 1.623 + 2.169 X$	0.136
	Mogra	1.037	0.189	5.697	$Y = 1.982 + 1.456 X$	0.309
	Rose	0.651	0.228	1.854	$Y = 1.827 + 1.754 X$	5.078
6h	Lavender	0.303	0.218	0.422	$Y = 1.242 + 2.546 X$	8.124
	Mogra	<b>0.295</b>	0.222	0.392	$Y = 0.908 + 2.777 X$	2.036
	Rose	0.503	0.224	1.127	$Y = 2.011 + 1.767 X$	5.748
24h	Lavender	0.196	0.164	0.234	$Y = 0.948 + 3.135 X$	1.182
	Mogra	<b>0.185</b>	0.156	0.219	$Y = 0.821 + 3.302 X$	2.524

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	Rose	0.339	0.188	0.611	$Y = 2.619 + 1.557 X$	2.487
48h	Lavender	<b>0.143</b>	0.118	0.173	$Y = 1.509 + 3.030 X$	1.644
	Mogra	0.147	0.122	0.177	$Y = 1.287 + 3.183 X$	1.255
	Rose	0.188	0.136	0.262	$Y = 2.928 + 1.636 X$	2.397

**NOTE:** LD<sub>50</sub>= Lethal Dose, 50% (express the dosage of a substance that is lethal to 50% of a population or test sample).

#### **Fumigation Toxicity:**

The doses of essential oil (Lavender, Mogra, and Rose) used in fumigant treatment were 4.074, 2.037, 1.018 and 0.509 µl/cm<sup>2</sup>. The results on the mortality of *T. castaneum* adults after 24 and 48 hour of exposure to Lavender, Mogra and Rose essential oils are presented in **Table 2**. LD<sub>50</sub> value of the lavender oil after 24 hour was 4.980 µl/cm<sup>2</sup> and after 48 hour of exposure period it became 3.180 µl/cm<sup>2</sup>. Though the LD<sub>50</sub> value were decreases by the time period so it's toxicity level was increasing. The lavender becoming more toxic at 48 hour of treatment for the test insect against *T. castaneum*. LD<sub>50</sub> value of the Mogra oil after 24 and 48 hour of exposure period were 7.339 and 5.463 µl/cm<sup>2</sup>. By the time period LD<sub>50</sub> value were decreasing and becoming toxic against *T. castaneum* and confirm higher mortality rate. LD<sub>50</sub> value of the Rose oil after 24 and 48 hour of exposure period were 4.031 and 2.252 µl/cm<sup>2</sup>. So after 48 hour of exposure time period the LD<sub>50</sub> value becoming low and confirm their increasing toxicity level. From above discussion it is clear that among the three essential oils viz Lavender, Mogra and Rose oil ; the Rose oil was relatively more toxic than other two oils compare to their LD<sub>50</sub> value. Among the three oils Rose oil shows highest mortality rate at the highest doses. After 48 hour of exposure time the LD<sub>50</sub> value of three oils were 3.180, 5.463 and 2.252 µl/cm<sup>2</sup>. So the highest toxicity rate compare to LD<sub>50</sub> value were Rose oil > Lavender oil > Mogra oil. Rose oil which didn't performed well in the contact treatment but well performed in fumigant treatment.

**Table 2:** LD<sub>50</sub>, 95% confidence limits and regression equation of Lavender, Mogra and Rose essential oils against adult *T. castaneum* after 24 and 48 hours of fumigation treatment.

Time of exposure	Oils	LD <sub>50</sub>	95% confidence limits		Regression equation	χ <sup>2</sup> value (at 2df)
			Lower	Upper		
			24h	Lavender		
	Mogra	7.339	2.872	18.751	Y = 2.045 + 1.614 X	0.377
	Rose	<b>4.031</b>	1.465	3.462	Y = 2.775 + 1.391 X	0.520
48h	Lavender	3.180	1.750	5.779	Y = 3.035 + 1.318 X	0.542
	Mogra	5.463	2.218	13.454	Y = 2.902 + 1.202 X	5.030
	Rose	2.252	2.100	7.740	Y = 3.088 + 1.421 X	0.139

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### Repellency:

Repellency assays showed mogra oil exhibiting significant effects ( $F = 25.25$ ,  $P < 0.001$ ) and achieving 100% repellency at  $0.0785 \mu\text{g}/\text{cm}^2$  within 3–4 hours, whereas lavender and rose oils showed lower and statistically insignificant effects.

**Table 3:** ANOVA of repellency for Lavender, Mogra and Rose essential oils against the larvae of *T. castaneum*.

Name of the species	Plant oils	Source of variation (SV)	SS	df	MS	F
<i>T. castaneum</i> larvae	Lavender	Between doses	3286.897	4	821.72	5.865
		Between time interval	449.317	4	112.32	0.801
	Mogra	Between doses	23646	4	5911.5	25.25**

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		Between time interval	213.2575	4	53.314	0.227
	Rose	Between doses	4231.781	4	1057.94	6.723
		Between time interval	470.9645	4	117.74	0.748

**NOTE:** SS= Sum of Squares, MS= Mean Square, df - Degrees of Freedom, F - F-ratio.\* = Significant at 5% level (P<0.05), \*\* = Significant at 1% level (P<0.01)

## DISCUSSION

The results of this study highlight the potential of Lavender (*Lavandula angustifolia*), Mogra (*Jasminumsambac*), and Rose (*Rosa damascena*) essential oils as effective biopesticides against *Triboliumcastaneum*, a major stored product pest. The progressive decline in LD<sub>50</sub> values over time for all three essential oils indicates their increasing toxicity with prolonged exposure, a critical characteristic for effective pest management. The LD<sub>50</sub> values for Lavender oil decreased significantly from 0.366 µl/cm<sup>2</sup> at 3 hours to 0.143 µl/cm<sup>2</sup> at 48 hours, making it the most toxic among the tested oils. Lavender oil's efficacy may be attributed to its high concentrations of monoterpenes, such as linalool and linalyl acetate, which are known to disrupt the insect nervous system and respiratory functions (Regnault-Roger et al., 2012). This aligns with previous research demonstrating the broad-spectrum insecticidal activity of lavender oil against pests like *Callosobruchusmaculatus* and *Sitophilus oryzae* (Adams et al., 2020). Mogra oil also showed a notable increase in toxicity over time, with LD<sub>50</sub> values dropping from 1.037 µl/cm<sup>2</sup> at 3 hours to 0.147 µl/cm<sup>2</sup> at 48 hours. Its insecticidal activity can be attributed to the presence of bioactive compounds such as benzyl acetate and jasmonates, which exhibit fumigant and contact toxicity properties (Kim et al., 2011). While less potent than Lavender oil, Mogra oil still demonstrated effective insecticidal properties, supporting its utility in integrated pest management (IPM). Rose oil, though the least toxic among the three, exhibited a similar trend, with LD<sub>50</sub> values declining from 0.651 µl/cm<sup>2</sup> at 3 hours to 0.188 µl/cm<sup>2</sup> at 48 hours. Compounds such as geraniol and citronellol, known for their insect repellent and toxic effects, are likely responsible for its activity (Isman, 2020). Although Rose oil's efficacy is comparatively lower, its potential for combination with other oils or as part of IPM strategies should not be overlooked. The findings from this study provide valuable insights into the fumigant toxicity of Lavender (*L. angustifolia*), Mogra (*J.sambac*), and Rose (*R.damascena*) essential oils against *Triboliumcastaneum*, a major pest of stored products. The decreasing LD<sub>50</sub> values over the 24- and 48-hour exposure periods demonstrate the potential of these essential oils as effective fumigants, with their toxicity

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increasing over time. Among the three oils, Rose oil exhibited the lowest LD<sub>50</sub> value (2.252 µl/cm<sup>2</sup> at 48 hours), making it the most toxic under fumigant conditions. This superior efficacy can be attributed to its high concentration of volatile bioactive compounds, such as geraniol and citronellol, which are known to have strong fumigant and insecticidal properties (Isman, 2020). Rose oil's performance in fumigant treatment, despite its relatively weaker efficacy in contact assays, highlights its potential in pest management strategies that rely on volatile modes of action. Lavender oil demonstrated a moderate toxicity, with LD<sub>50</sub> values decreasing from 4.980 µl/cm<sup>2</sup> at 24 hours to 3.180 µl/cm<sup>2</sup> at 48 hours. Lavender oil's effectiveness as a fumigant can be linked to its primary constituents, linalool and linalyl acetate, which have been shown to impair insect neurological pathways and respiration (Regnault-Roger et al., 2012). While it was the most toxic in contact assays, its performance in fumigant treatments was surpassed by Rose oil, emphasizing the role of specific application methods in determining efficacy. Mogra oil showed the highest LD<sub>50</sub> values (7.339 µl/cm<sup>2</sup> at 24 hours and 5.463 µl/cm<sup>2</sup> at 48 hours), indicating relatively lower toxicity compared to the other two oils. This reduced fumigant efficacy may stem from its composition, which includes benzyl acetate and jasmonates, compounds that are less volatile than those in Lavender and Rose oils. Nevertheless, its declining LD<sub>50</sub> values over time suggest a cumulative toxic effect with prolonged exposure, supporting its inclusion in integrated pest management (IPM) strategies (Kim et al., 2011). In repellency test showed mogra oil exhibiting significant effects, whereas lavender and rose oils showed lower and statistically insignificant effects against larval stage of *T. castaneum*. Another study on larval *T. castaneum* shows that Jasmine oil's repellency was insignificant between doses but significant between time intervals at a 5% level. Lemon grass showcased significant repellency at a 1% level between doses and a 5% level between time intervals. Sandalwood exhibited significant repellency at both 1% levels between doses and time intervals, emphasizing its robust deterrent effect against larval *T. castaneum*. (Haque and Faruki, 2024).

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## CONCLUSION

This study demonstrates the potential of Lavender, Mogra, and Rose essential oils as effective insecticides against *T. castaneum*, with their efficacy varying by application method and exposure time. Lavender oil was the most effective in contact treatments, while Rose oil excelled in fumigant assays. These findings provide a strong foundation for the development of sustainable pest management solutions using plant-based insecticides. Future research should focus on optimizing formulations, exploring synergistic combinations of these oils, and assessing their field applicability for sustainable pest management.

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Comment [K19]: *Triboliumcastaneum*

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